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ORIGINAL PAPER

Oocyte size distribution reveals ovary development strategy, number and relative size of egg batches in lumpfish (*Cyclopterus lumpus*)

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Abstract

The reproductive biology of fishes impact many other components of their life history, and can influence their vulnerability to fisheries, therefore for more informed management, a good understanding is essential. For lumpfish (*Cyclopterus lumpus*), a semi-pelagic species found across the north-Atlantic and targeted by fishers for their roe, comprehensive knowledge of this aspect of their life history is still lacking. Through a combination of regular sampling from scientific surveys and fisheries and modern methodology in fish reproductive biology, we investigated the ovary development of lumpfish throughout vitellogenesis. The results showed that ovaries of lumpfish had a wide range of oocyte sizes and that lumpfish are a determinate, batch spawner with ovary development taking at least 8 months. They spawn a maximum of two batches per season with a similar number of eggs in each batch. Unusually for a determinate batch spawner, the two batches were easily distinguished within the ovary prior to ovulation. Average egg size ranged from between 2050 and 2500 µm, with larger fish having larger eggs, and the egg diameter of the second batch being on average 1.6% smaller than the first. Lumpfish were documented as spawning over a 4-month period, but it is likely that spawning occurs over a greater period. A macroscopic and oocyte size frequency distribution (OSFD) scale for lumpfish is presented which can be used for future studies of lumpfish.

Keywords Lumpsucker · Maturation · Oocyte · Ovary development · Roe fishery

Introduction

The reproductive biology of fishes has a major influence on other biological aspects of the species in question, including growth, migration and predation risk. Energy allocated to spawning is no longer available for other processes such as growth. Feeding areas are often separate from spawning areas so fish must move between these areas, which entails an energetic cost, and may result in being exposed to an increased predation risk (Bentley et al. 2014). Many fishes form large spawning aggregations which can make them more vulnerable to fishing, and it may also be that they are only targeted by fishers when they are at the spawning area. Thus, knowledge on the reproductive biology of a species is essential for understanding its behaviour and many other aspects of its biology and can lead to more informed management of a commercial fish species (Lowerre-Barbieri et al. 2011).

Lumpfish (*Cyclopterus lumpus* L.) (pictured in Fig. 1) is a semi-pelagic species (Kennedy et al. 2016) found in the north-Atlantic and is most abundant in Arctic and sub-Arctic waters (ICES 2017). It begins life when it hatches from a nest of eggs which are guarded by the male. Juvenile Lumpfish may spend their first year inhabiting rock pools (Moring 1990) or associated with seaweed in coastal regions (Davenport and Rees 1993). As they grow, they migrate away from coastal areas out into the open sea, where they inhabit the pelagic zone (Holst 1993; Eriksen et al. 2014), before migrating back to the coast when they are close to spawning. It is at this point the females are the target of a commercial fishery for their roe.

The abundance of lumpfish around Iceland increases substantially during January–March. They are primarily found in areas on the north and west coast with only small numbers present on the south and south-east coast (Kennedy and Jónsson 2017). As the fishery for female lumpfish is a roe fishery, it targets fish with well developed gonads which are

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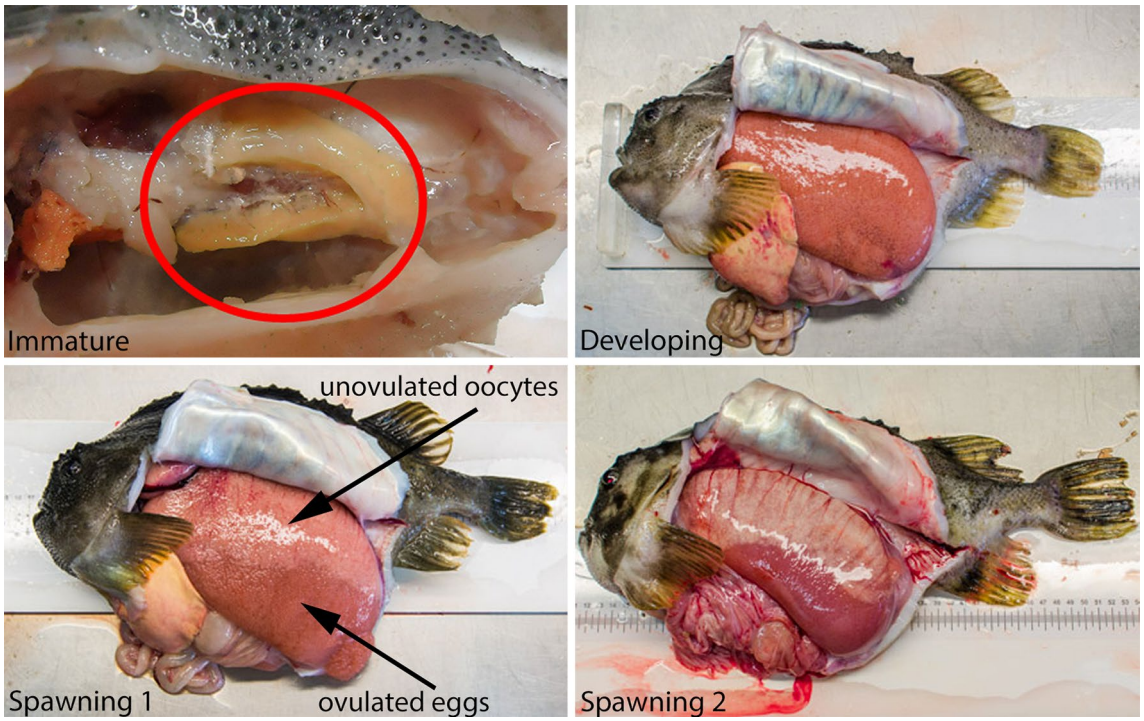

Fig. 1 Examples of macroscopic stages immature, developing, spawning 1 and spawning 2 for *Cyclopterus lumpus*. In immature stage, ovaries highlighted by red circle

presumably close to spawning. The fishing season gives an indication on when spawning may occur, however, without an examination of the gonads, it is not possible to know whether the fish have actually started to spawn.

Information on the timing of ovary development of lumpfish is sparse and many details asserted about its reproductive biology such as when they spawn, the length of the spawning season, whether it is a batch spawner and how many batches it spawns (Gregory and Daborn 1982; Davenport and Lønning 1983; Ehrenbaum 1904; Fulton 1907) are based upon anecdotal information and speculation, publications in the 'grey literature' or low sample sizes. There is also a lack of knowledge on the ovarian development organisation [synchronous, group synchronous, asynchronous (Murua and Saborido-Rey 2003)] and whether they exhibit determinate or indeterminate fecundity.

Unverified ageing of lumpfish indicates that females spawn for the first time when they are 3–4 years old (Hedeholm et al. 2014). Lumpfish produce 50–200 thousand eggs annually, depending on the size of the female, and in Greenland, this varies along a latitudinal gradient (Hedeholm et al. 2017). The ovaries and oviduct of lumpfish are fused to form a single sac which can fill up to two thirds of the body cavity (Davenport and Lønning 1983). Close to ovulation, an ovarian fluid is produced which fills the lumen of the oviduct. Upon ovulation, eggs fall into the lumen and are bathed in the ovarian fluid (Davenport and Lønning 1983). The role of the ovarian fluid remains unclear, but it has been suggested that is related to the inter-egg adhesion process which follows spawning (Davenport et al. 1983).

Macroscopic assessment of the gonads is the standard method for assessing maturity of fishes during surveys and sampling of commercial catches. When this is done, it is essential to have an established scale for classification of each stage. The descriptions of the stages should be clear, unambiguous and documented with images. To the author's knowledge, there is currently no such scale published for lumpfish.

Using a combination of modern methodology in fish reproductive biology (Thorsen and Kjesbu 2001; Witthames et al. 2009) and regular sampling of lumpfish from several sources (lumpfish and cod (*Gadus morhua* L.) gillnet fishery in Iceland and groundfish and pelagic surveys around Iceland) this study will investigate the reproductive biology of lumpfish. Specifically, examine the ovary development strategy of lumpfish, examine the range of egg sizes and document the occurrence of development stages throughout the year. This study will also establish a macroscopic and an oocyte size frequency distribution (OSFD) scale which can be utilised when collecting biological data on lumpfish, e.g. research surveys or port/market sampling, and to standardise terminology in future studies on lumpfish.

Materials and methods

Collection of samples

Lumpfish samples were collected from three scientific trawl surveys and the landings of two fisheries. The three trawl surveys were the Icelandic autumn and spring groundfish survey (hereafter referred to as autumn survey and spring survey, respectively) and the International Ecosystem Summer Survey of the Nordic Seas (ICES 2017) (hereafter referred to as the IESSNS survey) (Table 1; Fig. 2). The two fisheries were the commercial female lumpfish fishery (hereafter referred to as the lumpfish fishery) and cod gillnet fishery. Two fish which were bycatch from the monkfish (*Lophius piscatorius* L.) fishery in Breiðafjörður in November 2013 were also sampled. The samples collected from the fisheries were landed in three harbours: Skagaströnd, Stykkishólmur and Þórshöfn. The fish landed at these harbours are caught on the east of Húnaflói Bay, in Breiðafjörður and on the northern coast of the Langanes peninsula (Table 1; Fig. 2). Fish landed in Skagaströnd accounted for 92% of the fish sampled from the commercial fisheries. Lumpfish sampled from the

Table 1 The year, survey, time period in which samples were taken (D1, D2), the number of fish sampled (*n*), and minimum and maximum length of fish sampled during the study

Year	Survey	D1	D2	*n*	L.min	L.max
2013	Autumn	11/10	18/10	16	32	45
2013	Fishery (monk)	15/11	15/11	2	35	38
2014	Fishery (cod)	21/01	06/03	49	33	45
2014	Spring	01/03	16/03	50	34	49
2014	Fishery (lump)	09/04	03/07	71	30	47
2014	Autumn	04/10	17/10	30	32	47
2015	Spring	04/03	15/03	65	34	48
2015	Fishery (lump)	24/03	21/05	120	34	48
2015	Autumn	08/10	01/11	55	13	51
2016	Spring	26/02	18/03	93	34	49
2016	Fishery (lump)	21/03	29/05	221	34	48
2016	IESSNS	19/07	30/07	19	28	46

Fishery (lump) are samples from the female lumpfish fishery, fishery (cod) are samples from the cod gillnet fishery and fishery (monk) are samples taken from the monkfish fishery

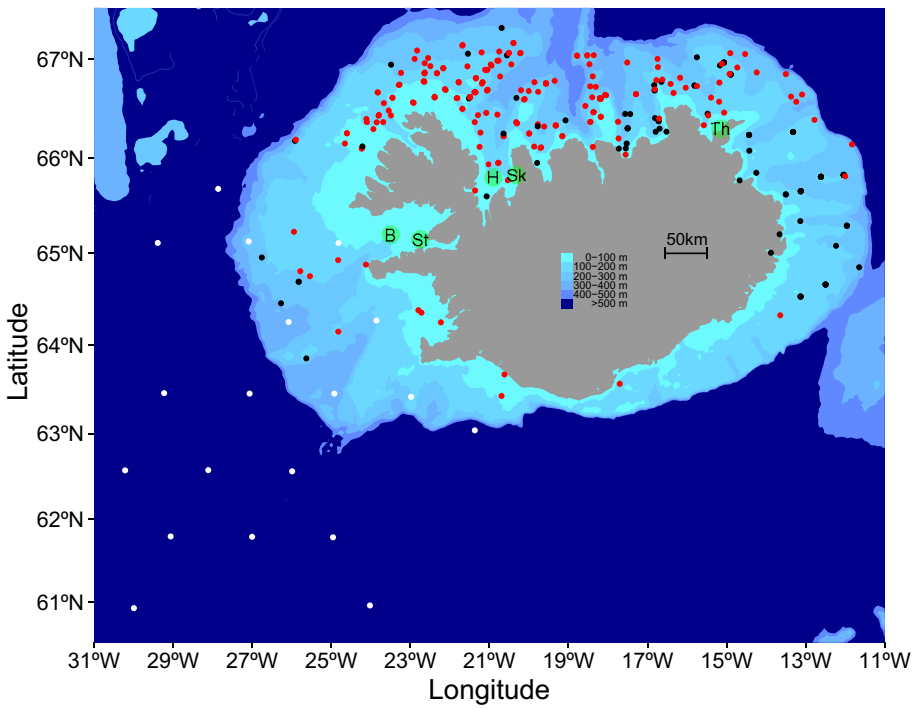

Fig. 2 Map showing location of sampling of *Cyclopterus lumpus*. The three harbours where fish were sampled from the fishery are shown (*St* Stykkishólmur, *Sk* Skagaströnd, *Th* Þórshöfn). The two bays mentioned in the text are marked (*B* Breiðafjörður, *H* Húnaflói). Points indicate stations where lumpfish were sampled during the Spring survey (red), Autumn survey (black) and the IESSNS survey (white)

lumpfish fishery were randomly selected from the catch upon landing. Lumpfish are a bycatch of the cod gillnet fishery and are usually discarded; upon request, any lumpfish caught were retained and landed for this study.

Fish caught during the surveys were sampled within 1 h of being caught, samples from the commercial fishery were covered in crushed ice and sampled within 24 h of being landed. Total length (nearest cm below) and total body, carcass (C_m), liver (L_m) and gonad (G_m) mass (precision ± 2 g) were measured for each fish. The mass of the stomach contents and mass of the stomach plus intestines were measured for the fish caught in the lumpfish and cod gillnet fishery. Each fish was assigned a macroscopic ovary stage from a maturity scale developed as part of the current study (Fig. 1; Table 2). Hepatosomatic (I_H) was calculated for each fish using the equation

$$I_H = L_m/C_m \times 100.$$

Total body mass was not used in the calculation of I_H due to the presence of large amounts of water and food (up to 800 g or 23% of total body mass) in many stomachs from the fish caught during the spring and autumn surveys.

Ovary sampling

Samples of ovary tissue were taken from each fish and stored in 45-ml tubes containing 10% buffered formalin. A preliminary study showed that ovaries at macroscopic stage 'developing' were homogenous in respect to oocyte diameter and oocyte samples for fish at this stage were considered representative of the whole ovary. For fish classified as developing, a single ovary sample of 4–6 g was taken from the caudal area of the ovary. In fish at stage 'spawning 1', the ovary is heterogenous with unovulated oocytes and ovulated eggs occupying separate areas of the ovary, these two areas are easily identified using the naked eye; unovulated oocytes are in the dorsal area of the ovary and ovulated eggs located in the ventral area of the ovary (Fig. 1). The unovulated oocytes and the ovulated eggs constitute two separate batches with the unovulated oocytes having a smaller diameter than the ovulated eggs (see results). Due to the heterogenous nature of the ovary at macroscopic stage spawning 1, two samples were taken and preserved in separate tubes; a sample of ovary tissue from the dorsal area of the ovary and a sample of ovulated eggs was also taken. For fish at macroscopic stage spawning 2, a sample of ovulated eggs and a section of ovary tissue was taken and preserved together in the same tube.

Measurement of oocytes

For each ovary sample, the ovary tissue was blotted to remove excess formalin and a small piece (sub-sample) was cut. The size of the sub-sample was dependent on visual assessment of the oocyte sizes. The oocytes were separated from the connective tissue using fine paintbrushes and photographed under a dissecting microscope; ×12.5 for the IESSNS and autumn survey samples and ×7 magnification for the spring survey and fishery samples. Light level was standardised using distilled water and grey level set at 207 ± 2. The images were analysed using ImageJ software (v. 1.49b, National Institute of Health, http://imagej.nih.gov/) and ObjectJ plug-in (v. 1.03 s, University of Amsterdam, http://simon.bio.uva.nl/objectj/) which measured the diameter of all oocytes present in the image. For each fish, all oocytes within the sub-sample were measured with the aim to measure a minimum of 150 oocytes > 400 µm. If the sub-sample did not contain 150 oocytes, an additional sub-sample of ovary tissue was taken and the above procedure repeated. A threshold of 400 µm was set for oocytes due to the low contrast between oocytes < 400 µm and the background thus the image analysis software could not reliably detect or measure these oocytes. In the case of ovulated eggs, a minimum of 80 were measured. Based upon the oocyte size distribution, the ovaries were assigned an OSFD maturity stage developed as part of the present study (Table 2).

Using the data from the image analysis two metrics were calculated for each ovary.

Average oocyte diameter For OSFD stage 2, this was the average diameter of all oocytes > 400 µm. For OSFD stages 3, 4 and 5, this was for all oocytes in the spawning group (SG) (Fig. 3), but did not include ovulated eggs. For OSFD 4 and 6, the average size of ovulated eggs was calculated.

Leading cohort oocyte (*LC*) *diameter* The average diameter of the largest 10% of oocytes. In OSFD stage 2, this was the largest 10% of all oocytes > 400 µm. In OSFD stages 3 and 5, this was the largest 10% of oocytes within the SG. For OSFD stages 4 and 6, this was the largest 10% of ovulated eggs.

LC diameter was used as an indicator of the progression of ovary development for OSFD stages 2 and 3 (Kjesbu 1994). For OSFD stage 3 fish (fish which did not contain ovulated eggs), the average diameter of batch 1 and batch 2 of the SG oocytes (Fig. 3g) and the ratio of the number of oocytes in each batch were calculated.

The effect of preservation on oocyte diameter was investigated using ovaries at OSFD stage 4 ($n = 14$). A sample of unovulated oocytes and ovulated eggs were taken from the ovary in the same manner as described above. An additional sample of unovulated oocytes and ovulated eggs was taken and placed in a tube without formalin. The samples which were not preserved in formalin were measured, within 4 h of being taken, in a comparable manner to samples preserved in formalin. The ovary samples which were preserved in formalin were measured a minimum of 4 weeks after preservation.

Table 2 Macroscopic maturity scale and accompanying oocyte size frequency distribution (OSFD) scale for lumpfish

Macroscopic stage	Description	OSFD stage	Description
Immature	The ovary is small and no developing oocytes are visible	1	Ovary contains only previtellogenic oocytes
Developing	The ovaries have increased in size and are easy to distinguish in the body cavity. Oocytes within the ovary are clearly visible. The ovary can be orange, purple or green. There may be a small amount of fluid close to the oviduct on the ventral area of the ovary, this should not be confused with the large amount of viscous ovarian fluid and ovulated eggs seen in the spawning stage	2	The oocyte size distribution shows a single group of oocytes within the ovary with almost all oocytes being < 1800 µm. The distribution is unimodal and as ovary development progresses, the distribution becomes negatively skewed
		3	A hiatus has formed around 1400-1600 µm separating the oocytes into two distinct groups. The larger group (by diameter, oocytes in this group are typically > 1800 µm) has a bimodal distribution, while the distribution of the smaller group is variable and occasionally may not be present
Spawning 1	There is a clear separation in the ovary; unovulated oocytes in the dorsal area of the ovary and ovulated eggs in the ventral area of the ovary. The unovulated oocytes are connected to the ovary tissue. The ovulated eggs look 'wet', are not connected to the ovary tissue, flow freely within the ovary and are bathed within a viscous ovarian fluid	4	The ovary contains unovulated oocytes and ovulated eggs. The ovulated eggs are larger than the unovulated oocytes. Both the ovulated eggs and unovulated oocytes have a unimodal size distribution. There may also be a group of smaller, unovulated oocytes < 1800 µm, the size frequency distribution of this group is variable
Partially spent	The ovary is similar in appearance to, and difficult to discern from, the developing stage	5	There may be one or two groups of oocytes present within the ovary. A larger group (by diameter, oocytes in this group are typically > 1800 µm) and has a unimodal distribution while the size frequency distribution of the smaller group (< 1800 µm) is variable and in a small number of cases may not be present
Spawning 2	The ovary is flaccid and contains mostly ovulated eggs which flow freely within the ovary. The ovulated eggs are not connected to the ovary tissue. They are bathed within a viscous ovarian fluid. The dorsal area of the ovary wall has a 'banded' appearance	6	The ovary will contain a single group of ovulated eggs which have a unimodal distribution. There may also be a group of smaller unovulated oocytes, the size frequency distribution of this group is variable
Spent	The ovary is flaccid, a small number of ovulated eggs may be visible	7	Ovary may contain a small number of ovulated eggs and (or) unovulated oocytes with a size distribution similar to unovulated oocytes from stages 2, 3, or 6

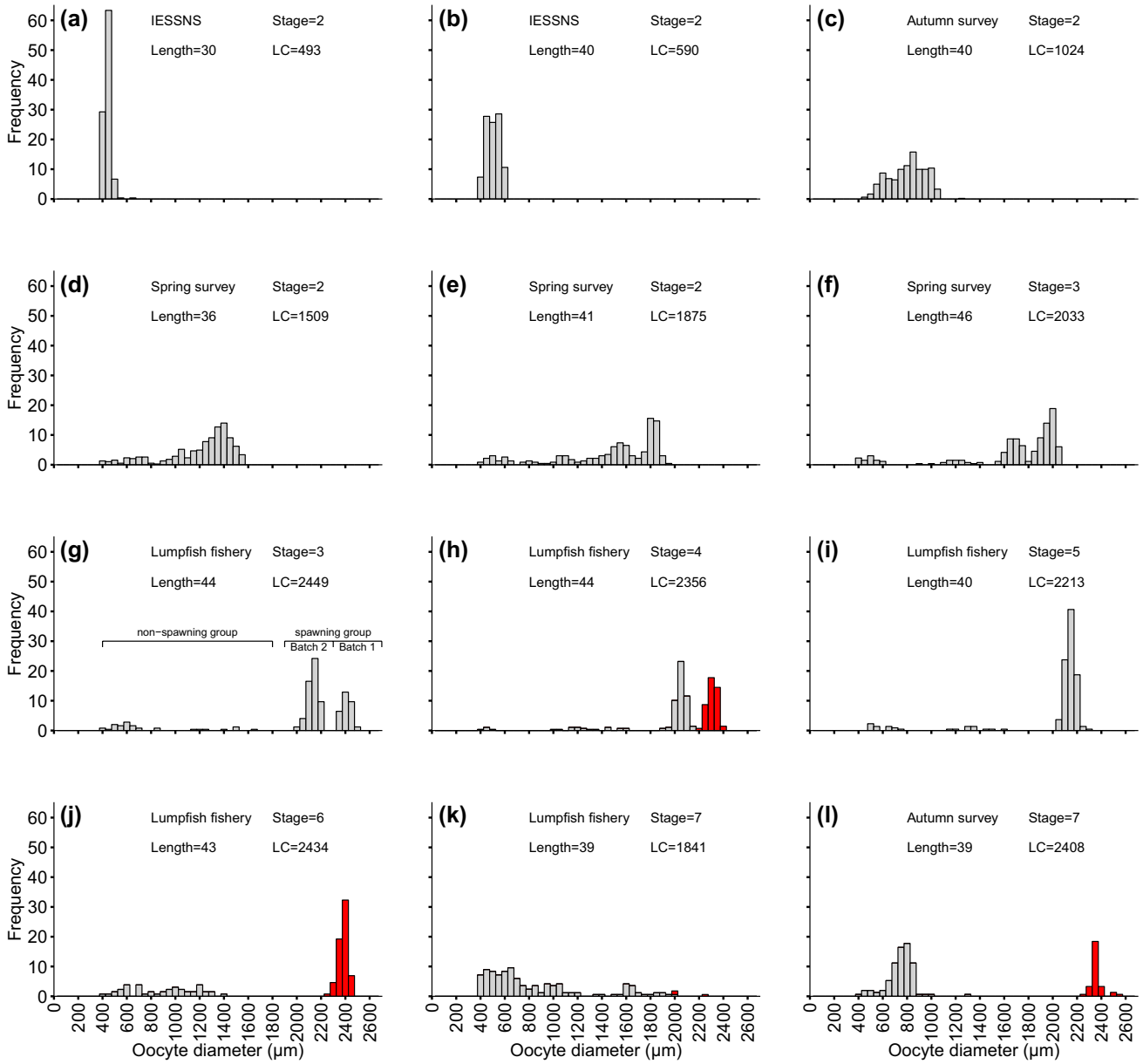

Fig. 3 Typical oocyte diameter frequency distribution of OSFD stages 2–7 of *Cyclopterus lumpus*. Leading cohort (LC) oocyte diameter (µm), length of the fish (cm) and the survey in which the fish was caught is shown. Unovulated oocytes are shown in grey, ovulated eggs are shown in red. Ovulated and unovulated oocytes were sampled separately, thus frequency distribution reflects distribution of sizes within but not between the two types

The storage of fish on ice for up to 24 h, which kept fish at a low temperature without freezing, was considered unlikely to have a significant impact on oocyte diameter.

Statistics

Statistical analysis was carried out using the 'R' program [version 3.3.2. R Core Team (2015)]. Linear segmented regression of log-transformed data was used to examine the change in I_H through ovary development (using LC oocyte diameter as a proxy). The effect of preservation on the size of oocytes was tested using Student's t test. The effect of batch number and C_m on ovulated egg size was investigated using linear regression, with oocyte diameter and C_m log transformed.

Results

Macroscopic maturity and OSFD scales

Based upon macroscopic characteristics and the results of the OSFD analysis, a 6-stage macroscopic maturity scale was developed (Table 2; Fig. 1). All macroscopic stages except the partially spent stage could easily and rapidly be distinguished using the naked eye.

The oocyte size frequency distribution of fish classified as developing, began as unimodal (Fig. 3a) but as ovary development progressed, the distribution became negatively skewed with a continuous distribution of oocytes from the smallest to largest oocytes (Fig. 3b–e). When the LC oocyte diameter was approximately 2000 µm, a hiatus formed at around 1400–1600 µm, creating two groups. The group of smaller oocytes was termed the non-spawned group (NSG), as these oocytes would not be spawned (see Discussion), and the group of larger oocytes was termed the spawning group (SG) (Fig. 3f–g) as they would be spawned in the current spawning year. Shortly before the hiatus formed, what is to become the SG begins to exhibit a bimodal distribution (Fig. 3e). After the hiatus has formed between the NSG and SG (Fig. 3f), a hiatus formed within the SG splitting the SG into two smaller groups. Each of these groups had a unimodal distribution (Fig. 3g) and were termed batch 1 (larger diameter) and batch 2 (Fig. 3g). Of the 313 fish classified as OSFD stage 3 fish, no individuals were seen with more than 2 batches within the SG.

For fish at OSFD stage 4, the oocyte distribution of the unovulated oocytes had two groups of oocytes which corresponded with the NSG and SG from macroscopic developing stage (Fig. 3h). However, there was only a single batch of oocytes in the SG. The ovulated eggs had a unimodal distribution and were larger in diameter than the SG. The ovulated eggs corresponded with batch 1 of the SG of the developing stage. Of the 115 fish at OSFD stage 4, no fish were seen to have more than 1 group of oocytes within the unovulated SG oocytes.

Twenty-four fish, initially classified as developing, had only a single group of oocytes in the SG (Fig. 3i). These are fish which had presumably spawned their first batch of eggs and were classified as OSFD stage 5 and thus should have been initially classified as partially spent.

The size distribution of the ovulated eggs of OSFD stage 6 fish was unimodal (Fig. 3j). This would correspond with the unovulated oocytes of fish at OSFD stage 5. Some of the ovaries of fish at OSFD stage 6 contained an unovulated NSG.

The size distribution of oocytes in stage 7 was variable and dependent on when the fish was caught. If caught in the autumn survey, oocytes < 1800 µm tended to have a similar distribution of those from stage 2. If caught in the fishery, the distribution of oocytes < 1800 µm tended to be much wider than stage 7 fish caught in the autumn survey.

Based upon this information on oocyte diameter distributions, a 7-stage OSFD scale was developed (Table 2). OSFD stages 2, 3 and 5 were difficult to distinguish macroscopically, therefore fish classified as developing could be at OSFD stages 2, 3 or 5.

Ovary stages

Of all the fish analysed during this study, the majority were at OSFD stage 3 (Table 3). All fish sampled during the IESSNS survey had begun ovary development and were classified as developing. Two fish were at OSFD stage 3 which indicates that they would spawn in the current year (Figs. 4, 5). One fish had recently spawned, but development for the following year's spawning had not begun.

In the autumn survey, all fish except one was classified as developing (Fig. 4, 5). Nine of the fish showed signs of past spawning (small number of ovulated eggs were present in the ovary), but all of these fish had begun ovary development for the following year and had a similar oocyte diameter to OSFD stage 2 fish also caught during the autumn survey.

The majority of the fish caught in the cod gillnet fishery or during the spring survey were at OSFD stage 2 with some at stage 3 (Fig. 4, 5). One fish at OSFD stage 1 and one at stage 7 were caught during the spring survey. The stage 7 fish was identified by a small number of ovulated oocytes but given the number and size of the developing oocytes (mean OD = 968 µm), this fish would likely spawn in the current year and the ovulated oocytes appear to be left over from the previous year´s spawning. The fish caught in the lumpfish fishery were composed mostly of fish at OSFD stages 3 and 4 with some fish at stages 2, 5, 6 and 7 (Fig. 4, 5). Spawning fish (fish at OSFD stages 4 and 6) were caught during weeks 13–23 in Húnaflói Bay and in week 27 in Breiðafjörður (Fig. 5).

Table 3 Number of fish used in the study at each stage of the oocyte size frequency distribution scale (OSFDS)

Stage	1	2	3	4	5	6	7
Number	2	296	313	115	24	28	13

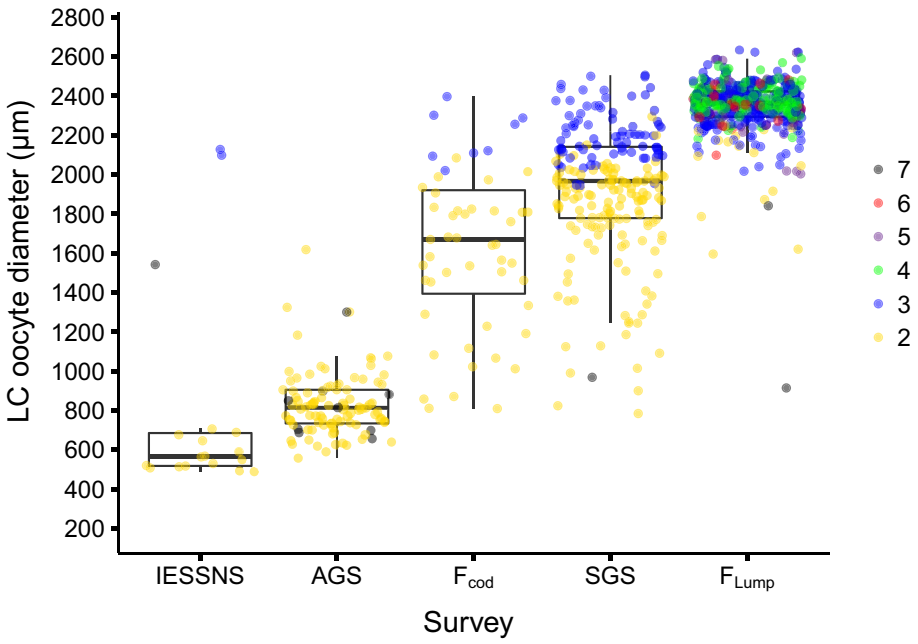

Fig. 4 Distribution of LC oocyte diameters and OSFD stages for all lumpfish from each survey and fishery (all years combined). *IESSNS* International Ecosystem Summer Survey of the Nordic Seas, *AGS* Autumn groundfish survey, *SGS* Spring groundfish survey, F_{cod} cod gillnet fishery, F_{lump} lumpfish fishery. Legend shows OSFD maturity stages

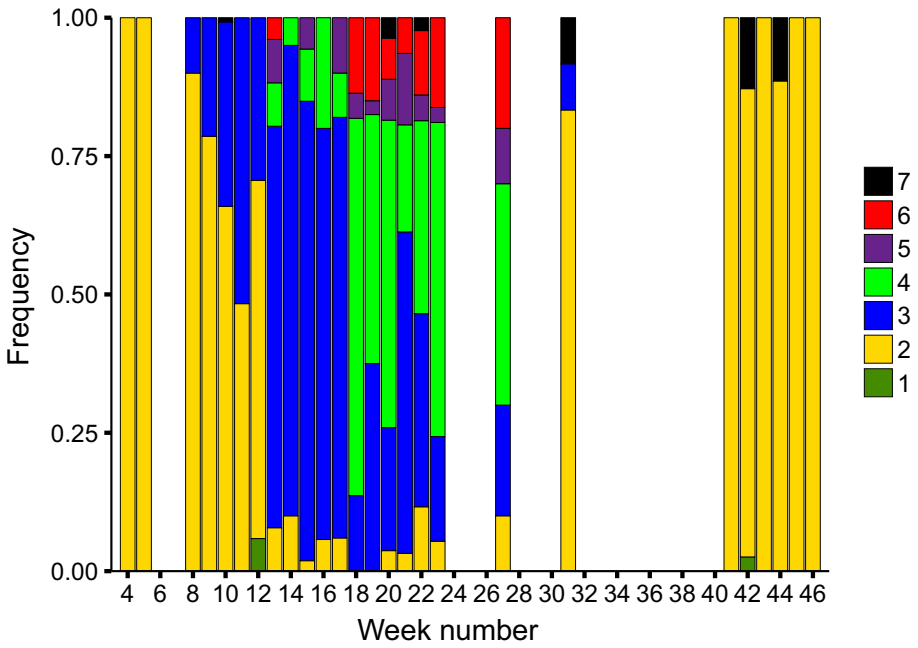

Fig. 5 Proportion of lumpfish at each OSFD stage versus week number. All surveys/fisheries and years combined. Legend shows OSFD stages

Hepatosomatic index

Segmented regression of log-transformed I_H and LC oocyte diameter revealed breakpoints at 821 and 1929 µm (segmented linear regression, $R^2 = 0.35$, $p < 0.0001$). In the initial and second segment of the regression, I_H was positively correlated with LC oocyte diameter with the initial segment being steeper than the second. In the terminal segment, there was a negative correlation between I_H and LC oocyte diameter (Fig. 6).

Oocyte and egg sizes

Preservation had no significant effect on average oocyte diameter of unovulated oocytes (Student's t test, $t = 0.92$, $df = 25.97$, $p = 0.37$) or ovulated eggs (Student's t test, $t = 0.72$, $df = 25.17$, $p = 0.48$). Ovulated egg size was significantly different between fish at OSFD stage 4 and stage 6 (Student's t test, $t = 2.18$, $df = 39.91$, $p = 0.04$) (Fig. 7); ovulated eggs from stage 4 ovaries were 1.6% smaller than those from stage 6. Log-transformed ovulated egg size was significantly correlated with log-transformed C_m and

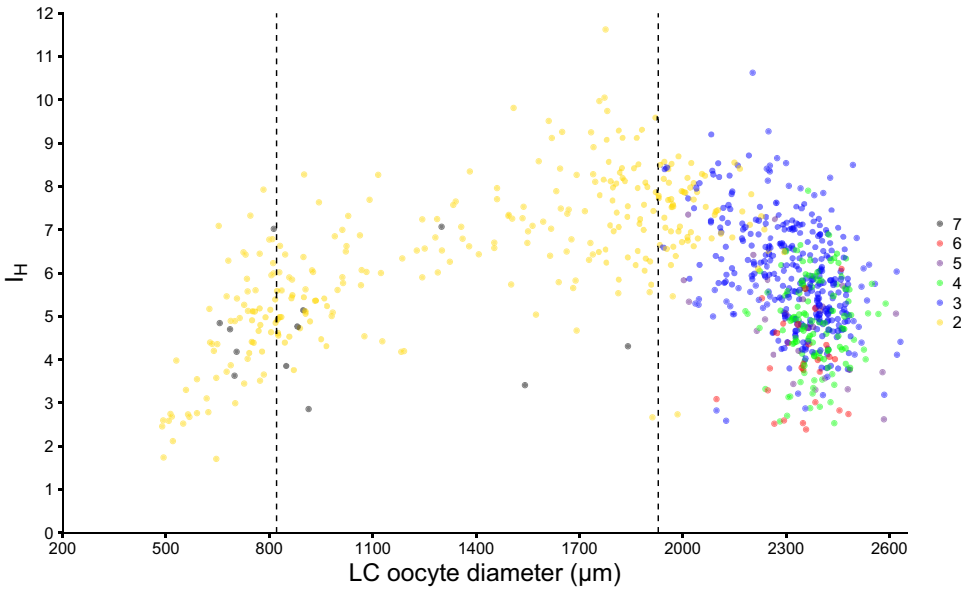

Fig. 6 I_H versus LC oocyte diameter for OSFD stages 2–7. Dashed lines show breakpoints from the segmented regression and legend shows OSFD stages

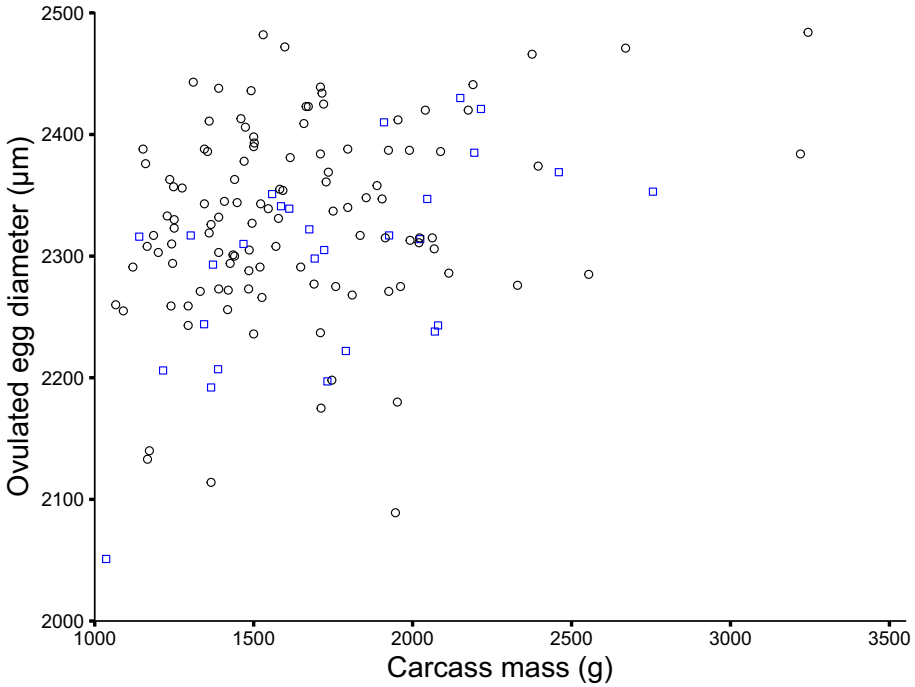

Fig. 7 Ovulated egg diameter versus carcass mass (C_m) for *Cyclopterus lumpus* at OSFD stage 4 (black circles) and 6 (blue squares)

negatively correlated with batch number (linear model, $R^2 = 0.13$, $p < 0.0001$) (Fig. 7).

The average ratio of the number of oocytes in batch 1 and batch 2 of the SG of OSFD stage 3 fish was 0.50 ± 0.09 (mean ± SD), $n = 297$. Average size of unovulated oocytes in OSFD 4 fish were positively correlated with (linear regression, $R^2 = 0.65$, $p < 0.0001$), and significantly smaller than (Student´s t test, $t = -15.65$, $df = 211.62$, $p = < 0.0001$), ovulated eggs. The mean oocyte diameter of the unovulated batch was on average, 8.2% smaller than the ovulated batch.

Discussion

Ovary development strategies among teleosts are diverse (Murua and Saborido-Rey 2003; Smith and Wooten 2015) and have been documented in a wide range of species including fish from the families Clupeidae (Kurita et al. 2003), Gadidae (Skjæraasen et al. 2010), Gobiidae (Teichert et al. 2014), Merlucciidae (Korta et al. 2010), Pleuronectidae (Nichol and Acuna 2001), Soleidae

(Witthames and Greer Walker 1995), Scombridae (Greer Walker et al. 1994) and Xiphiidae (Poisson and Fauvel 2009). The present study has uncovered an unusual characteristics for a determinate batch spawner which adds to this diversity, i.e. multiple batches of oocytes being clearly distinguishable within the ovary before ovulation. In addition, some of the characteristics uncovered are quite typical, but not universal, for teleosts such as larger fish producing larger eggs and egg size decreasing with batch number (Hinckley 1990; Buckley et al. 1991; Kjesbu et al. 1991; Kennedy et al. 2007).

The current study was hindered by the lack of histological examinations of the ovaries, this would have been particularly useful for investigating the ovary development organisation which was not possible. A wide range of oocyte sizes were found to be present within the ovaries of lumpfish, but without histological examination it was difficult to identify the development stage of these oocytes. In group-synchronous ovaries, there is a population of oocytes which are fairly synchronous and will be spawned during the next spawning opportunity. In contrast, in ovaries which exhibit asynchronous development, oocytes of all stages of development are present without dominant populations (Murua and Saborido-Rey 2003). Oocyte size can give an indication of the development stage (oocytes at a later development stage are generally bigger), but this is affected by the presence of atretic oocytes which decrease in size as the oocyte is broken down and reabsorbed. It may be that for lumpfish at OSFD stage 2, there was a narrow range of oocyte development stages and the large range in oocyte sizes is the result of numerous atretic oocytes at various stages of reabsorption. Such a scenario would fit the description of group-synchronous development. However, the large range in oocyte sizes may well be a result of asynchronous development. Given that relative fecundity decreased as ovary development proceeded (Kennedy, unpublished data), indicates that atretic oocytes were indeed present throughout OSFD stage 2 and group-synchronous is more likely; however, without histological confirmation this currently remains unclear.

The question arises what happens to the oocytes in the NSG after the formation of the hiatus. In Greenland halibut and numerous species of Antarctic fishes, multiple groups of oocytes are present within the ovary with one batch being used for the next spawning event and another being used for a spawning event approximately one year later (Shandikov and Faleeva 1992; Everson 1994; Kennedy et al. 2011). Many of the ovaries at OSFD stage 6 did not contain an NSG indicating that these oocytes are reabsorbed through atresia and not used for future spawning events; further studies utilising histology should be used to confirm this.

As a hiatus formed between the SG and the NSG before ovulation occurred shows that lumpfish are determinate spawners, i.e. maximum annual fecundity is set before spawning occurs. With the presence of two distinct batches of oocytes within the SG and that each batch is ovulated separately, it is reasonable to conclude that lumpfish are batch spawners, producing two batches per spawning season. It is unusual for separate batches to be distinguishable within the developing cohort of oocytes of a determinate batch spawner. Oocyte batches usually arise from a homogeneous group of vitellogenic oocytes with a unimodal size distribution (Kjesbu et al. 1990; Nichol and Acuna; 2001; Kennedy et al. 2007). It is generally referenced that lumpfish spawn 1–3 batches of eggs which appears to come from Ehrenbaum (1904). The author did not have access to this reference and so unable to examine the background to this claim but as it is a fish identification book, it seems unlikely to document a direct observation. Of all the fish at OSFD stages 3 and 4 examined (a total of 428 fish), there was no evidence that the fish would spawn more than two batches, i.e. no with fish with 3 batches within the unovulated oocytes for OSFD stage 3 or with 2 batches within the unovulated oocytes for OSFD stage 4. It would thus appear that for lumpfish to spawn more than two batches is unusual, if at all. It is more difficult with the current data to assess whether producing only a single batch per year is a regular occurrence. Using the OSFD scale developed as part of this study, fish producing a single batch would be classified as OSFD stage 5 or 6 (depending on whether ovulation had occurred or not) as they would be indistinguishable from fish which had already spawned one batch. In order to investigate whether OSFD stage 5 fish had already spawned one batch or would only spawn a single batch in the current year, a histological analysis is required in order to investigate the presence or absence of post-ovulatory follicles; the presence of post-ovulatory follicles is considered the most reliable evidence of previous spawning activity in fishes.

With the two batches being easily distinguishable before ovulation, it was straightforward to estimate the relative size of each batch, something which usually requires tank experiments with live fish (Kjesbu et al. 1991). With the average ratio of large to small oocytes being 0.50 leads to the conclusion that the number of eggs in each batch is similar. Unfortunately, the current data do not allow us to estimate the duration between batches; there appears to be only a single direct observation of the time between batches which is 13 days (Fulton 1907).

I_H was positively correlated with LC oocyte diameter until the LC oocyte diameter was approximately 1900 µm. This follows a similar pattern to that seen in another determinate spawner, plaice *Pleuronectes platessa* L. 1758 (Kennedy et al. 2007). The liver contains significant levels of lipids and is important for energy storage, but is also the organ in which vitellogenin is produced. It is difficult to discern how much of this increase in liver weight is a result of energy intake being stored in the liver and how much is

a result of the remobilisation of nutrients from other components of the body for production of vitellogenin. Many of the fish sampled from the lumpfish fishery had empty stomachs indicating feeding decreases when they are close to spawning. Thus, the final phase of ovary development is mostly funded using accumulated reserves and explains the decrease in liver weight after the LC oocyte diameter reaches approximately 1900 µm.

Given that the difference in diameter between the unovulated oocytes and the ovulated eggs within the same fish was greater than the difference between the ovulated eggs from OSFD stage 4 and stage 6 fish indicates that the second batch of oocytes continues to develop within the ovary after the first batch has been ovulated. Therefore, total investment in the ovary is not complete, even when the first batch of eggs has been ovulated.

The diameter of the majority of ovulated egg batches fell within a range of approximately 300 µm (2200–2500 µm), which concurs with previously published data on egg sizes for this species (Fulton 1907; Russell 1976). This variation between individuals is similar to that found in other fish species (Gisbert et al. 2000; Vallin and Nissling 2000; Kennedy et al. 2007). Ovulated egg size increased with fish size and decreased from the first to second batch. The effects of fish size and batch number on egg size are well documented in other species (Buckley et al. 1991; Kjesbu et al. 1991; Kennedy et al. 2007). Larvae from larger eggs tend to be larger and have greater yolk reserves (Blaxter and Hempel 1963; Marteinsdóttir and Steinarsson 1998; Rideout et al. 2005) upon hatching, but the degree to which this impacts survivorship to the adult stage is still not fully understood.

Lumpfish has a long vitellogenic period with ovary development for the following year's spawning having begun by the end of July. The first spawning individuals were caught in late March which demonstrates that ovary development must take at least 8 months, with the true length currently unknown as it is unclear when ovary development actually started. Lumpfish were documented as spawning over a 4-month period with individuals with ovulated eggs being caught from week 13 until week 27. The spawning season is likely to be longer as the collection of samples was limited by the timing of the fishery, with the fishery closing in 6 of the 7 lumpfish fishing areas by mid-June. The lumpfish fishery continues in Breiðafjörður until the end of July, and early August in some years, but logistics limited the ability to sample fish. Given that this is a roe fishery suggests that lumpfish close to spawning are present until this time. Two fish were also caught in week 31 (first week of August) which were close to spawning (LC oocyte diameter approximately 2100 µm) so they would presumably have spawned in August.

There was a notable lack of fish at OSFD stage 5 with the reason for this being unclear. It may be that after spawning, lumpfish move away from the shallow areas in which the fishery takes place while they wait for the final maturation and ovulation of their second batch, so are less likely to be caught by the fishery. There is greater fishing effort at the beginning of the season (Marine and Freshwater Research Institute, unpublished data), so it may be that a large portion of the OSFD stage 3 and 4 fish are caught in the fishery, leading to a low proportion of OSFD stage 5 fish in the population. It is also interesting that very few OSFD stage 2 fish were caught in the lumpfish fishery, but were abundant in the spring survey and the cod gillnet fishery which preceded the fishery by 2–3 weeks. Some fish would obviously move from OSFD stage 2 to OSFD stage 3 in this time. Given the large range in LC oocyte diameters of fish caught in the spring survey and the cod gillnet fishery and the large drop in the proportion of OSFD stage 2 fish from week 12–13 would suggest this is not the full explanation. As the fishery occurs in shallow water (5–50 m depth) and the spring survey primarily catches lumpfish from 20–300 m depth (Kennedy and Jónsson 2017), lumpfish which are close to spawning move to shallow water whereas fish which are not, remain in deeper water. The fishery therefore selects for fish close to spawning. With a higher fishing effort at the beginning of the season, early spawning fish may be facing a higher fishing mortality in comparison with fish spawning later in the season which could have negative effect on the diversity of genetic and(or) phenotypes within the lumpfish population (Consuegra et al. 2005).

From the results of this study, a macroscopic maturity scale is proposed that can be utilised during sampling of lumpfish. Our photos present a clear visual guide with a description of each stage which can be printed and laminated for use on research or fishing vessels. Unfortunately, it was not possible to get a photo of a spent fish during the study. We aim to capture an image as soon as possible and the author can be contacted in the future if one is needed and one has been obtained. The scale partially followed the terminology of Brown-Petersen et al. (2011); however, we split the spawning capable stage into three sub-stages: spawning 1, partially spent and spawning 2. Given that lumpfish spawn only two batches, the progression through spawning can be easily identified when ovulated eggs are present within the ovary. When collecting data on maturity of lumpfish, the extra data obtained by splitting this stage is likely to prove useful in future studies. The main problem when assessing the maturity stage of lumpfish macroscopically is the inability to reliably discern the developing and partially spent stages. In order to avoid the classification of developing fish as spawning capable (Brown Petersen et al. 2011), depending on the aims of the study being carried out, it may be prudent to drop the partially spent stage. Given that partially spent fish are infrequently observed, the potential for misclassification is greater when this stage is

included. Indications of past spawning in fish caught during the autumn survey were frequently missed during the macroscopic assessment, indicating that careful examination of the gonads is needed. If specifically looking for indications of past spawning, it is wise to take samples back to the laboratory for measurement of oocyte sizes in order to reliably assess the proportion of fish exhibiting signs of past spawning.

In summary, lumpfish are a determinate, batch spawner which spawn no more than 2 batches of eggs per season. Average egg size ranges from between 2050 and 2500 µm, with larger fish having large eggs. The number of eggs spawned in the second batch is similar to the first batch, but the egg diameter of the second batch is approximately 1.6% smaller. In Iceland, fish were documented as spawning over a 4-month period but it is likely that spawning occurs over a greater period than that recorded during the present study.

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Compliance with ethical standards

Conflict of interest The author declares that he has no conflict of interest.

Human and animal rights All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

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